


**REMARKS**

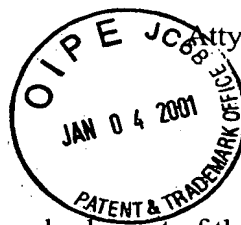
This amendment is made to conform the application with the attached Sequence Listing.  
A copy of the Notice to Comply is attached herewith. In the event that this paper is not timely filed, Applicants respectfully petition for an appropriate extension of time. The fees for such an extension or any other fees which may be due with respect to this paper, may be charged to Deposit Account No. 50-1067.

Respectfully submitted,

4 January 2001

  
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Don J. Pelto  
Reg. No. 33,754

McKenna & Cuneo, L.L.P.  
1900 K Street, NW  
Washington, DC 20006  
Telephone: 202.496.7500  
Facsimile: 202.496.7756

**Specification Amendments, 3 January 2001**

Page 5, lines 6-21 (Amended)

Figure 1 depicts an overview of a preferred embodiment of the single-phase amplification methods of the present invention. PolyA<sup>+</sup> or total RNA is annealed with the single-stranded oligo dT-tailed promoter primer, T<sub>7</sub>T<sub>20</sub> (ggC Cag TgA ATT gTA ATA CgA CTC ACT ATA ggg Agg Cgg (T)<sub>20</sub> (SEQ ID NO. 1)), creating a primer-template mixture. First strand cDNA synthesis is accomplished by combining the first strand cDNA reagent mix (Superscript II, buffer, DTT, and dNTPs) with the primer-template mixture and incubating at the appropriate time and temperature. Second strand cDNA synthesis is then performed by mixing the first strand cDNA reaction with second strand reagent mix, containing secondary cDNA mix (depc-H<sub>2</sub>O, Tris-HCl (pH7.0), MgCl<sub>2</sub>, (NH<sub>4</sub>)SO<sub>4</sub>, beta-NAD<sup>+</sup>, and dNTPs) and cDNA enzyme mix (Amplitaq DNA polymerase, *E. coli* ligase, *E. coli* RNase H, and *E. coli* DNA polymerase I), followed by incubation at the appropriate times and temperatures. The resulting double-stranded (ds) cDNA contains a functional T7 RNA polymerase promoter, which is utilized for transcription. Finally, in vitro transcription is performed by combining the (ds) cDNA with IVT reagent (NTP, buffer, T7 RNA polymerase), yielding amplified, antisense RNA.

Page 13, lines 11-20 (Amended)

Step 1: Primer-template annealing. The HPLC purified primer may be obtained from a -20°C storage stock, prepared in a 100 µM solution with TE (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA (pH 8.0)) and diluted 1:1 with glycerol (for a final concentration of 50 µM in 50% glycerol and 50% TE). Where the desired nucleic acid sample is poly(A)<sup>+</sup> RNA, a T<sub>7</sub>T<sub>20</sub> primer (ggc cag tga att gta ata cga ctc act ata ggg agg cgg (T)<sub>20</sub> (SEQ ID NO. 1)) (Operon Technologies, Inc., Alameda, California) for example, may be used. In such case, an RNA sample (10 to 100 ng mRNA or 1-2 ug total RNA suspended in 2.5 µl or less) can be mixed with 0.5 µl primer to give a final volume of 3 µl. The mixture can be incubated at 70°C for 5-10 minutes, then cooled to 4°C.